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Human Leukocyte antigen and classical Hodgkin lymphoma

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Chapter 1



General introduction
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HUMAN LEUKOCYTE ANTIGEN

The major histocompatibility complex (MHC) was first identified in mice and was shown to be responsible for the rejection of transplanted tumors across different strains (1). For this work George Snell was awarded the Nobel Prize in Physiology or Medicine in 1980. In humans, the MHC proteins are commonly referred to as human leukocyte antigens (HLA). The HLA genes reside in a ~3500 kb segment on 6p21.31 (Figure 1) and this is the most polymorphic gene region in mammals, with some genes having more than 2,000 alleles (Table 1). The HLA locus is composed of three regions, i.e. HLA class I, II, and III (2).

HLA class I molecules are present on the surface of all nucleated cells, where they present peptides derived from the cytosol to circulating CD8⁺ T cells. The HLA class I cell surface molecule is a heterodimer, consists of an alpha chain encoded by the classical class I genes (HLA-A, HLA-B, and HLA-C) and one stabilizing β 2-microglobulin chain. Exons 2 and 3 of the HLA class I genes encode the highly polymorphic amino acid residues clustering within the peptide binding cleft of the HLA alpha chain. The HLA class I locus also contains the less polymorphic non-classical HLA-E, HLA-F and HLA-G genes (3–6).

HLA class II molecules are expressed on the surface of professional antigen presenting cells (APC) including B cells, macrophages and dendritic cells. They primarily present exogenously derived peptides (from bacteria, toxins etc.) to circulating CD4⁺ T cells. The HLA class II heterodimer consists of an alpha and beta chain encoded by polymorphic A1 and a variable number of B genes respectively, i.e. HLA-DRA1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1. The most polymorphic regions are within the peptide binding cleft of the HLA class II molecules. The HLA class II locus also contains the less polymorphic non-classical HLA-DM, HLA-DO, transporter associated with antigen presentation protein 1 or 2 (TAP1/2) and TAP binding protein (TRBP also called tapasin) genes (3–6).

The HLA class III region contains genes that are not related to the HLA class I and class II genes. This gene-dense region contains among others critical mediators of innate immunity such as tumor necrosis factor (TNF), heat shock proteins (HSP) and complement proteins (7,8). The HLA region is characterized by a high degree of linkage disequilibrium (LD). This means that alleles at different loci are co-inherited more frequently than is expected by chance. The combination of alleles that are inherited together are referred to as haplotypes (9,10).

For both the HLA class I and the HLA class II molecules, the polymorphic amino acid residues in the binding groove dictate specificity for antigenic peptides, such that each HLA allele can present a restricted and unique repertoire of antigenic

peptides. The HLA molecule also interacts with specific residues of the T cell receptor (TCR). Different populations exhibit different frequency distributions of the HLA alleles and can also have specific haplotypes (9,11,12).

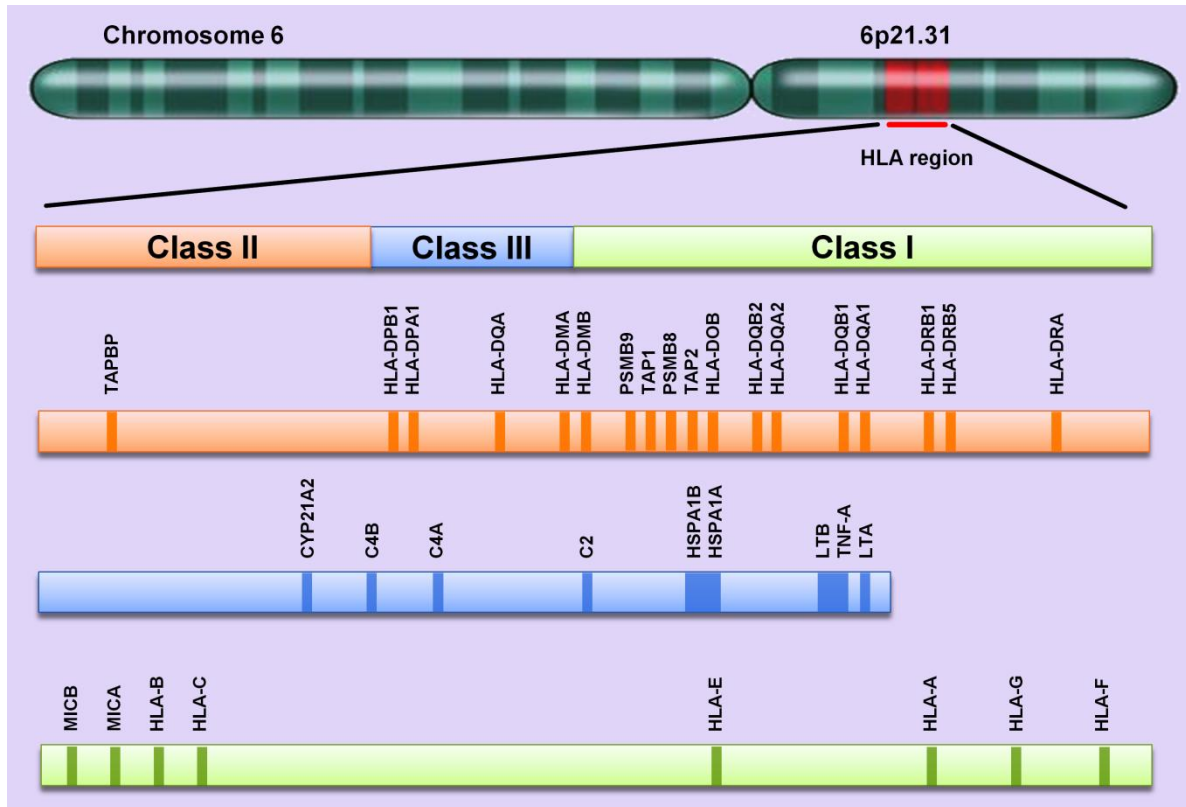


Figure 1. The HLA region and the location of the HLA genes. The HLA region is conventionally divided into three subregions: I, II, and III. Each subregion contains numerous genes. Of the class I and II genes, only the main genes are depicted. C2, C4A and C4B; complement components 2, 4A and 4B. CYP21A2; cytochrome P-450, subfamily. HSPA1A, HSPA1B, and HSPA1L; heat-shock protein 1A A-type, B-type and 1A-like. LTA and LTB; lymphotoxins A and B. MICA and MICB; major-histocompatibility-complex class I chain genes A and B. P450; cytochrome P-450. PSMB8 and PSMB9; proteasome β 8 and 9. TAP1 and TAP2; transporter associated with antigen processing 1 and 2. TAPBP; TAP-binding protein (tapasin). TNF-A; tumor necrosis factor α .

The role of HLA in immune responses

Presentation of antigenic peptides by HLA is a pivotal mechanism in induction and maintenance of adaptive immune responses that are orchestrated by T cells (13). These T cells have been strictly selected in the thymus to only recognize foreign, non-self antigenic peptides. The antigenic peptides for HLA class I presentation are derived after ubiquitination and proteolytic degradation of cytosolic defective ribosomal products (DRiPs) or from viral proteins (14,15). The 8-9 amino acids long peptides are delivered into the endoplasmic reticulum (ER) by TAP1/2 and loaded onto the HLA class I heterodimer with the help of chaperons such as tapasin and calreticulin (16–18). This stable HLA class I-peptide complex will then be presented

on the cell surface for recognition by CD8⁺ T cells. Upon recognition, CD8⁺ T cells proliferate and differentiate into cytotoxic T cells (CTLs) that secrete interferon γ (IFN γ), granzyme and perforin which can kill the antigen presenting cell. This CTL response is central to antiviral defense during acute infection. Some activated CD8⁺T cells differentiate into memory cells that can quickly reactivate at re-infection (Figure 2A).

HLA class II is usually restricted to professional APCs, but it can be conditionally expressed in non-APCs such as epithelial cells upon IFN γ stimulation (19). HLA class II molecules are associated with the invariant chain (Ii) in the endosomal vesicle which harbors engulfed extracellular proteins and digested peptides (20). HLA-DM enables the proteolytic cleavage and replacement of Ii with antigenic 12-16 amino acids long peptides (21,22). The stable HLA class II-peptide complex can potentially be recognized by an antigen specific naïve CD4⁺ T cell and induce cytokine production, proliferation and differentiation into effector and memory T cells. These activated CD4⁺ T cells may differentiate into effector Th1 cells that secrete IFN γ which augments CTL responses. Alternatively, they may differentiate into T helper 2 (Th2) cells that secrete interleukin (IL) 4, IL5 and IL13 and stimulate B cells to differentiate into antibody producing plasma cells. In general Th2 cytokines can suppress Th1 differentiation and vice versa. Some of the activated CD4⁺ T cells may differentiate into Th17 cells, which secrete IL17 and promote inflammation through neutrophils (Figure 2B) (23,24).

Table 1. The number of HLA class I and HLA class II alleles.

HLA class I		HLA class II			
Gene	Alleles	Gene	A-allele	B-allele	AxB
HLA-A	1,519	HLA-DP	28	145	4,060
HLA-B	2,069	HLA-DQ	35	144	5,040
HLA-C	1,016	HLA-DR	3	966	2,898
HLA-E	10	HLA-DM	4	7	28
HLA-F	22	HLA-DO	12	9	108
HLA-G	46				

Data from European Bioinformatics (EBI) server IMGT/HLA Database v 3.15.0., retrieved on February 2014 available at <http://www.ebi.ac.uk/ipd/imgt/hla/>.

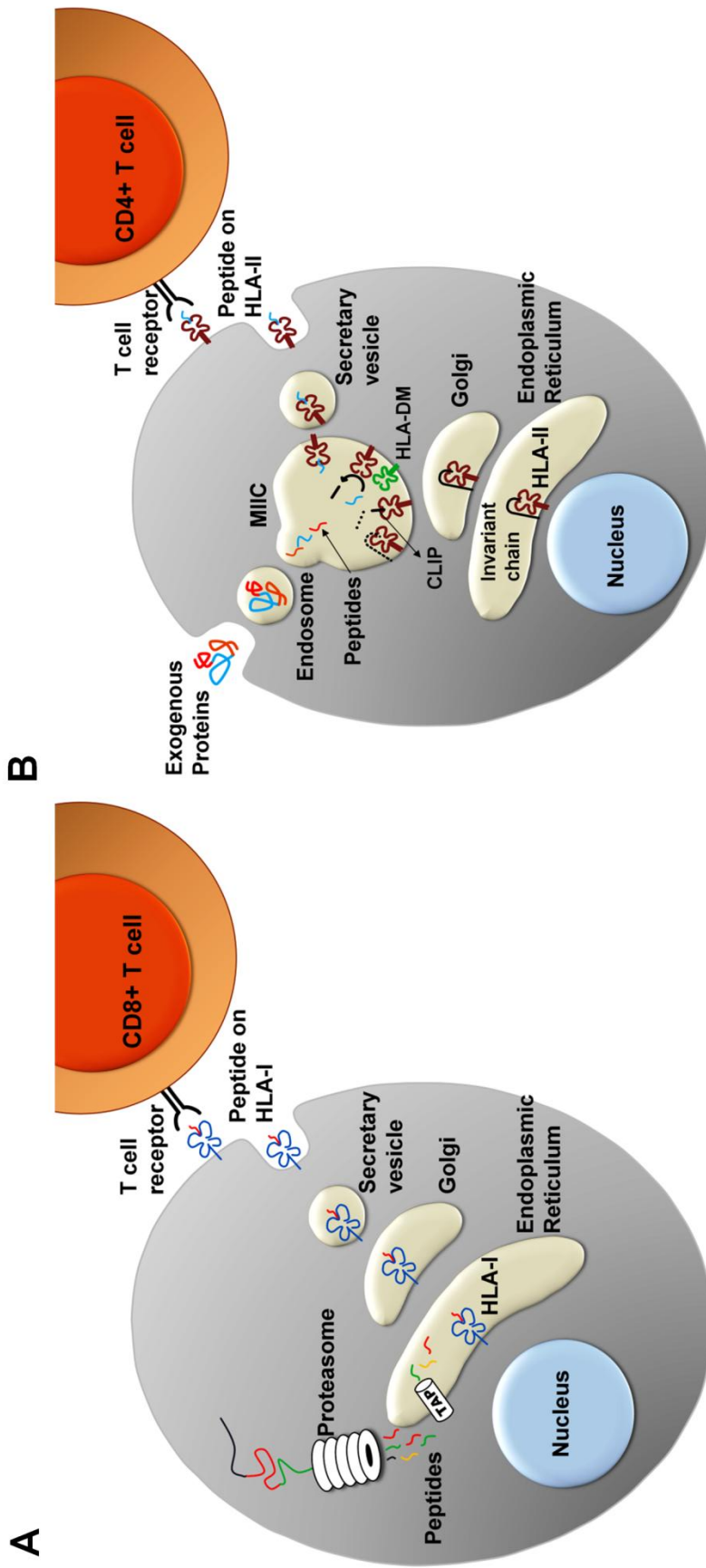


Figure 2. Schematic representation of endogenous (HLA class I) and exogenous (HLA class II) antigen processing and presentation.

(A) Endogenous proteins undergo proteolytic degradation by proteasome in the cytosol. The resulting peptides are translocated via transporter associated with antigen presentation (TAP) into the endoplasmic reticulum and these peptides then loaded on to HLA class I (HLA-I) β -2 microglobulin heterotrimeric complex. Peptide-HLA-I complex are released and transported via the Golgi to the plasma membrane presentation of peptide to CD8+ T cells. (B) Exogenous proteins are imported into the cell. The assembly of HLA class II and invariant chain occur in the endoplasmic reticulum. Both endosomes consisting endocytosed protein and Golgi vesicle containing HLA-II-invariant chain heterotrimeric complex fuse to form MHC class II compartment (MIIC). In MIIC the endocytosed proteins and Ii are degraded by resident proteases. The class II-associated Ii peptide (CLIP) fragment is exchanged for an antigenic peptide with the help of the dedicated chaperone HLA-DM. Peptide-HLA-II complex is then transported to the plasma membrane for the presentation of peptides to CD4+ T cells.

HLA and disease susceptibility

The HLA class I and class II gene products are critical in the regulation of immunity against various infections, autoimmunity and cancer. Selection of antigenic peptides is dependent on the allelic variants of the HLA molecule. Both the peptide and the HLA molecule influence the recognition by the T cell receptor and determine the strength and nature of the immune response (25). It is not surprising that HLA is a main genetic risk factor in more than 50 infectious diseases, autoimmune diseases and cancers (26).

The number of studies on associations of HLA alleles with lymphoma is limited. The most notable HLA associations are observed for follicular lymphoma with an increased risk observed for HLA-DRB1*01:01 and a decreased risk observed for DPB1*03:01 and HLA-DRB1*13 (27). HLA-DRB1*04:01 is a risk allele for diffused large B cell lymphoma (28).

HODGKIN LYMPHOMA

Hodgkin lymphoma (HL) was first described in 1832 by Thomas Hodgkin as a disease of the lymphatic system (29). Clinical features of HL include asymptomatic, painless lymphadenopathy usually in the cervical area, mediastinum or abdomen. Depending on stage and clinical risk factors, 65%–90% of patients can be rendered disease-free after five years with current treatment strategies (30). Treatment involves multiple cycles of chemotherapy (ABVD or BEACOPP) sometimes followed by radiotherapy (31). Long term survivors frequently suffer from treatment related adverse effects, such as cardiac disease and secondary malignancies (32).

HL can be subdivided into two main subtypes, i.e. classical Hodgkin lymphoma (cHL) which accounts for 95% of all cases, and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). HL is characterized by the presence of large neoplastic cells, known as Hodgkin Reed Sternberg (HRS) cells, in a background of reactive immune cells (33). Based on the composition of the microenvironment, cHL can be classified into Nodular sclerosis (NS), Mixed cellularity (MC), Lymphocyte rich (LR) and Lymphocyte depleted (LD) subtypes (34). NLPHL is composed of vague nodules of numerous reactive lymphocytes admixed with large popcorn-shaped tumor cells called lymphocyte predominant (LP) cells. Unlike HRS cells, the LP cells of NLPHL are CD15 and CD30 negative, while positive for the B cell marker CD20 (35).

HRS cells are large mono, bi- or multi-nucleated cells with prominent nucleoli. The phenotype is unusual with expression of CD30 and CD15 and loss of CD45, the B cell receptor (BcR), CD20 and multiple B cell specific transcription factors (36,37). Based on the presence of immunoglobulin gene rearrangements and the frequent presence of crippling somatic mutations in the immunoglobulin genes it is clear that

HRS cells are of germinal center B cell origin (38). In the normal B cell maturation process B cells with a low affinity or non-functional BcR undergo apoptosis during negative selection in the germinal center. However, HRS precursor cells escape from apoptosis probably due to a combination of multiple factors. Constitutive activation of the nuclear factor κ -light-chain-enhancer of activated B cells (NF κ -B) is a hallmark of HRS cells and provides one of the main pro-survival signals (39). Activation of CD30, CD40, receptor activator of NF κ -B (RANK) and Notch contribute to the constitutive activation of the NF κ -B pathway in HRS cells (39–42). In addition several genetic aberrations such as amplification of the avian reticuloendotheliosis viral oncogene homolog (c-Rel) locus (43), mutations of NF κ -B inhibitor (I κ B α) (44) and tumor necrosis factor, alpha-induced protein 3 (TNFAIP3 or A20) (45) can contribute to the activation of NF κ -B pathway.

The reactive cells that are abundantly present in cHL play a crucial role in the pathogenesis of cHL. The T cells that form rosettes around the HRS cells have a T helper 2 (Th2), T regulatory (Treg) phenotype (46,47). It is generally accepted that the HRS cells need these cells to survive and HRS cells produce various cytokines and chemokines to specifically attract these cells. Chemokine ligand 5 (CCL5) and CCL17 (also known as TARC) are involved in attracting Th2 cells and Tregs. HRS cells secrete cytokines IL4 and IL13 that skew the immune response from a Th1 to a Th2 type reaction, thus changing immunosurveillance and promoting HRS cell survival (48). In addition, IL13 is an autocrine growth factor, which provides survival signals to HRS cells by binding to IL13R expressed on the HRS cell surface. It is also known that HRS cells produce immune suppressive cytokines like IL10 and TGF β to suppress CTLs (49). IL13, tumor necrosis factor alpha (TNF α) and tumor growth factor beta (TGF β) attract fibroblasts and promote fibrosis, a characteristic feature of the NS subtype of cHL.

Epstein Barr virus

More than 90% of adults worldwide harbor lifelong latent EBV infection in a small fraction of their B cells (50). In general, primary EBV infection is asymptomatic, but in a proportion of the individuals it manifests as infectious mononucleosis. Although latent EBV infection persists throughout life, the chance of an individual to develop an EBV related malignancy is low due to effective EBV specific CTL responses. A history of infectious mononucleosis is associated with an increased risk (~3x) of developing EBV associated cHL (EBV+ cHL) despite normal immune surveillance (51,52).

In EBV+ cHL cases, a clonal infection with EBV is observed in HRS cells indicating that infection with EBV is an early step during transformation (53). HRS

cells show an EBV latency type II infection pattern, that is characterized by expression of latent membrane protein 1 (LMP1), LMP2A and EBV nuclear antigen 1 (EBNA1) (54). LMP1 mimics activated CD40 and contribute to the activation of NFκ-B. LMP2A mimics the BcR and provides anti-apoptotic signals to the infected B cells (55). The impact of EBV status on clinical outcome of cHL is somewhat controversial, although there is consensus on adverse prognosis in older patients (56–58).

HLA expression by HRS cells

HLA class I expression by HRS cells is observed in 70% of the EBV+ cHL and in 14% of the EBV- cHL cases (59). Downregulation of HLA class I may help to avoid anti-tumor responses against tumor derived antigenic peptides. In EBV+ cHL cases the retention of HLA class I expression is enigmatic, as HLA class I restricted immune responses to either tumor or EBV derived antigenic peptides should be detrimental to the HRS cells. Although the EBV latency type II proteins expressed by HRS cells are not immunodominant, they can induce immune responses in both healthy individuals and HL patients (60). It is very likely that HLA class I polymorphisms modify the immune response in EBV+ cHL patients. Studies from our group and subsequent studies in the West European cHL population have convincingly shown that HLA-A*01 carriers are at risk, while HLA-A*02 carriers are protected against the development of EBV+ cHL (51,61). It is known that HLA-A*01 is less efficient in presenting EBV latency type II proteins, whereas HLA-A*02 can efficiently present such peptides and induce effective CTL responses (62,63). Expression of HLA class II is observed in the HRS cells in about 50% of the cHL cases, irrespective of EBV status. Downregulation of HLA class II by HRS cells has been shown to be an adverse prognostic factor (64).

Incidence of cHL and association with EBV, age and subtype

The age adjusted incidence rate of cHL is 3.5 in males / 2.9 in females per 100,000 in Caucasians, 2.9 males / 1.6 females per 100,000 in Hispanics and 1.4 males / 1.0 females per 100,000 in Asian countries. In general there is a male predominance of 1.3 to 1 (65). The prevalence of positive EBV status is variable with ethnic and geographical differences (66). Up to 87% of Hispanic cHL patients have EBV+ HRS cells (67), in Caucasians EBV is present in 20-40% of the cHL cases (68) and in Orientals the percentage is intermediate (69,70). EBV positivity shows a male predominance and is associated with the MC subtype (71). A distinguishing epidemiological feature of cHL is its bimodal age incidence curve with a prominent age peak at the third decade and a second peak at the seventh in developed countries (Figure 3). In less developed countries the first peak slightly moves towards

a younger age (67,69). CHL in children is usually associated with EBV positivity and the MC subtype. In young adults, cHL is associated with EBV negativity and the NS subtype. At older age, MC and EBV positivity are more common (68,72–74).

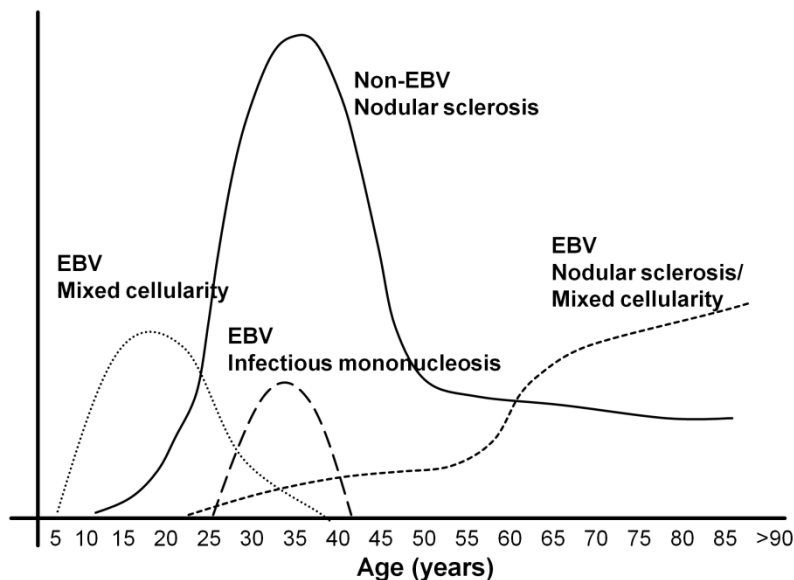


Figure 3. Four disease classical Hodgkin lymphoma model considering age, EBV and subtype (adapted from A. Armstrong et al., 1998 (72)). The graph shows four superimposed cHL disease subgroups based on EBV status, subtype and association with infectious mononucleosis (symptomatic primary EBV infection in adolescence).

AIM OF THIS THESIS

The aim of this thesis is to study associations of HLA alleles and single nucleotide polymorphisms in the HLA region with cHL and in cHL subgroups stratified by EBV and age at diagnosis. It is expected that genetic variants responsible for predisposition to or protection against cHL will be different between these subgroups and will allow for a better understanding of the underlying immunopathogenetic mechanisms. In **chapter 2** the current knowledge on genetic associations with cHL susceptibility, disease prognosis and treatment related secondary toxicities and malignancies is reviewed. Both targeted gene approach studies including HLA and non-HLA genes and genome wide screening studies are discussed. In **chapter 3**, HLA alleles are studied to identify associations with cHL and EBV stratified subgroups. To explore HLA associations in different age ranges in an unbiased approach, HLA typing data from multiple West European cHL populations from the Netherlands, Denmark, Sweden and Scotland were pooled in **chapter 4**. As it is difficult to use predefined age subgroups, we applied a novel methodology based on a moving average. In **chapter 5**, HLA-A*01 and HLA-A*02 carrier frequencies in an EBV stratified cHL cohort from Brazil is studied to establish if the associations observed in multiple Western European cHL populations are also present in the Brazilian population.

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